

# Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell

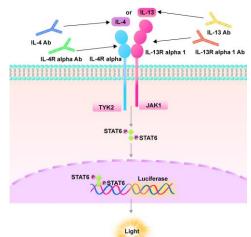
Catalog No.	Size
CHEK-ATF075	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

## • Description

The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell with endogenous IL-4 R alpha and IL-13 R alpha 1 expression was engineered to express STAT6 signaling response element. When stimulated with human IL-4 or IL-13 protein, receptor-mediated signaling can drive STAT6-mediated luminescence. Neutralization of biological effect of human IL-4 and IL-13 protein by corresponding antibody results in a decrease in luminescence.

## • Application

• Screen for neutralizing antibodies blocking the stimulation of human IL-4 and IL-13 protein.



Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell

### • Cell Line Profile

Cell line	Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL)
Incubation	37°C with 5% CO <sub>2</sub>
Doubling Time	22-24 hours
Transduction Technique	Lentivirus



## • Materials Required for Cell Culture

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-II)
- CO<sub>2</sub> Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

#### • Recovery

- 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
- 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
- 3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
- 4. Discard the supernatant and resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
- 5. Incubate at  $37^{\circ}$ C with 5% CO<sub>2</sub> incubator.



#### • Subculture

- Remove and discard culture medium.
- 2. Wash the cells once with sterile PBS.
- 3. Add 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached.
- 4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
- 5. Add appropriate aliquots of the cell suspension to new culture vessel.
- 6. Incubate at 37°C with 5% CO<sub>2</sub> incubator.
  - **Subcultivation Ratio:** A subcultivation ratio of 1:6 to 1:10 is recommended.
  - **Medium Renewal:** Every 2 to 3 days.

### Cryopreservation

- 1. Remove and discard spent medium.
- 2. Detach cells from the cell culture flasks with 0.25% trypsin.
- 3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
- 4. Resuspend the cell pellets with complete growth medium and count viable cells.
- 5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of  $5\times10^6$  to  $1\times10^7$  cells/mL.
- 6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a -80°C freezer overnight, then transferring to liquid nitrogen storage.

### • Storage

Product format: Frozen

• Storage conditions: Liquid nitrogen immediately upon receipt



## • Signaling Bioassay

## **Human IL-4 Protein Stimulation (RLU)**

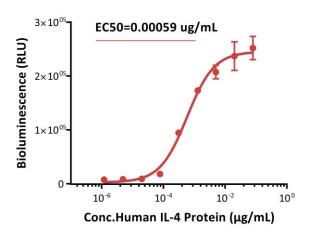
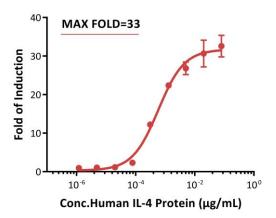


Fig1. Response to human IL-4 protein (RLU). The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-4 protein (Cat.No.IL4-H4218). The EC50 was approximately  $0.00059 \, \mu g/mL$ .

#### **Human IL-4 Protein Stimulation (FOLD)**



**Fig2. Response to human IL-4 protein (FOLD).** The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-4 protein (Cat.No.IL4-H4218). The max induction fold was approximately 33.



### **Human IL-13 Protein Stimulation (RLU)**

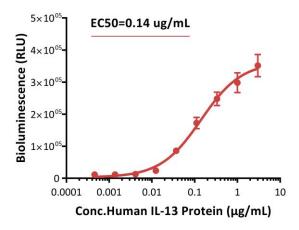
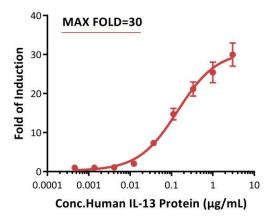


Fig3. Response to human IL-13 protein (RLU). The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-13 protein (Cat.No.IL3-H52H4). The EC50 was approximately  $0.14 \,\mu\text{g/mL}$ .

#### **Human IL-13 Protein Stimulation (FOLD)**



**Fig4. Response to human IL-13 protein (FOLD).** The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-13 protein (Cat.No.IL3-H52H4). The max induction fold was approximately 30.



## • Application

#### Anti-human IL-4 R alpha Neutralizing Antibody Screening

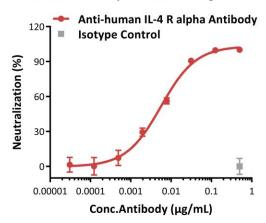


Fig5. Inhibition of human IL-4 protein-induced reporter activity by anti-human IL-4 R alpha neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-4 protein (Cat.No.IL4-H4218) with a final concentration of  $0.002 \, \mu g/mL$ . The EC50 of anti-human IL-4 R alpha neutralizing antibody is approximately  $0.0054 \, \mu g/mL$ .

#### Anti-human IL-4 R alpha Neutralizing Antibody Screening

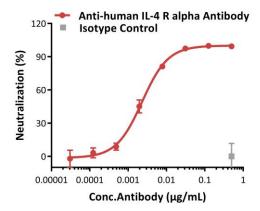
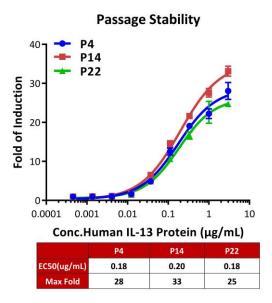


Fig6. Inhibition of human IL-13 protein-induced reporter activity by anti-human IL-4 R alpha neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-13 protein (Cat.No.IL3-H52H4) with a final concentration of  $0.1 \mu g/mL$ . The EC50 of anti-human IL-4 R alpha neutralizing antibody is approximately  $0.0023 \mu g/mL$ .



#### Passage Stability



**Fig6. Passage stability analysis by Signaling Bioassay.** The continuously growing Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-13 protein. Human IL-13 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 4-22.

#### • License Disclosure

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#### • Related Products

<u>Products</u>	<u>Cat.No.</u>
Human IL-4 Protein, premium grade	IL4-H4218
Human IL-13 Protein, His Tag	IL3-H52H4